

**METHODS FOR DETERMINING A NUCLEOTIDE AT A SPECIFIC  
LOCATION WITHIN A NUCLEIC ACID MOLECULE**

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**Abstract of the Disclosure**

Novel methods for determining the existence or nonexistence of a test nucleotide on a strand of DNA are provided. The methods involve the use of a proofreading  
10 polymerase that is capable of incorporating a labeled nucleotide in a primer into and extension product if there is a match between the test nucleotide on the strand of DNA and the complementary nucleotide on the primer, but which excises the labeled nucleotide and does not incorporate it into an extension product if there is a mismatch. The presence or absence of the test nucleotide then may be established by determining  
15 whether the label is preserved or lost following the reaction. Methods involving the use of a quencher-chromophore pair are also provided.

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